The thymus and myasthenia gravis.

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An introduction to clinics and pathophysiology of Myasthenia Gravis (MG) is given in **Chapter I**. The varying involvement of individual muscles, the role of antibodies to the acetylcholine receptor (a-AChR) and possible mechanisms for the induction of MG are discussed. An outline of the study is given.

Reviewing the literature (**Chapter IIa**) it can be learned that thymectomy (TE) is generally considered as an effective therapy in Myasthenia Gravis (MG) but that opinions about indications for TE are quite divergent. The way of assessing the effect-rate of TE deserves critical notes in some of the studies on this subject. The duration of MG before TE, the presence of a thymoma and the way of operation seem to be important factors of influence on the effect of the operation. No improvement of MG after incomplete removal of a thymus and good results after rethymectomy plea for performance of TE by surgeons with ample experience of this operations. The risk of postoperative MG-related complications are a further argument for concentration of TE to specialized centres. The period between the operation and clinical effect of TE varies from patient to patient; most patients do not improve within the first six months after TE. General recommendations concerning perioperative management are summarized in this chapter.

Our own experience with 100 patients is described in **chapter IIb**. No relations between patient factors and the effect of TE were found, except for the fact that severely diseased patients derived less benefit from the operation, as was found by others, and that patients with thymoma derived considerably less benefit from TE. A latent period between operation and start of improvement was also found in our own group. Because the clinical course in those patients benefitting from TE was very stable and generally no further improvement was seen three years after the operation we defined benefit by TE as improvement of at least two points on a disability scale or complete remission after three years without the influence of immunosuppressive drugs and this definition was used in the rest of the study. Our perioperative protocol is described in IIb.

Arguments exist for a possible relation between HLA-phenotype of the patient and effect of TE. We studied this relation in a group of 20 responders and 20 non-responders (**chapter IIc**) but found the patient's HLA-type not to be indicative for the effect of TE in MG.

Clinical course and changes in serum levels of antibodies to the acetylcholine receptor (a-AChR) were reported in 82 patients with myasthenia gravis during a period of 1-8 years after TE in **chapter IIIa**. Decrease in a-AChR immediately after TE was influenced by changes in total IgG. Immunosuppressive medication lowered serum a-AChR at all time points. In a subgroup of 41 patients without thymoma who had no immunosuppressive drugs there was a steady decrease in a-AChR concomitant with clinical improvement from 6 weeks after TE and afterward.

a-AChR serum levels are partly a result of antibody production by peripheral blood lymphocytes (PBL). Spontaneous (SSA) and PWM-stimulated (PSA) in vitro production of a-AChR by PBL were measured in 79 patients with generalized MG as described in **chapter IIIb**. SSA was only found in non-thymomatous early onset MG, and correlated with a poor clinical response to TE (TE). PSA was found in patients who had not yet been thymectomized or who

had derived no benefit from TE. Furthermore, the use of immunosuppressive medication did not affect PSA. We suggested that measuring PSA after TE might be helpful in the decision for rethymectomy.

Sixteen patients were followed before and soon after TE in **chapter IIIc**. PSA disappeared in all patients, at least temporarily, between 6 weeks and 1 year afterwards, independent of the clinical course and eventual clinical effect of the operation. A recurrence was found only in one of the five patients who derived no benefit from the operation.

There was no correlation between the changes in PSA and serum levels of a-AChR suggesting a minor role of circulating PBL and the thymus in the total production of a-AChR.

The findings from chapter IIIb and IIIc support the hypothesis that the therapeutic effect of TE can be explained by a removal of a source of autoreactive lymphocytes.

The predictive value of a moderate improvement and antibody-changes at several time points after TE were analyzed in **chapter IIId**. Both parameters had a considerably predictive value for the eventual effect of TE; a combination of both parameters was most useful in case of an unfavourable clinical course.

The role of anti muscle antibodies (AMA) in the diagnosis of a thymoma in patients with Myasthenia Gravis (MG) is evaluated in **chapter IVa**. A comparison was made between an ELISA and Western Blot assay for antibodies to citric acid muscle extract (a-CAE) and an immunofluorescence assay (IF). Sera from 234 selected MG-patients and 123 controls were tested. There was no essential difference between the ELISA and IF. The Western Blot was superior in early onset patients but less useful in patients with an onset beyond the age of 40 years. Unusually high post-test probabilities were found by our criteria of patient selection which seem most realistic for clinical practice. The Western Blot revealed no differences in specificity of AMA in thymoma and non-thymoma patients irrespective of age at onset of disease.

Chapter IVb describes the frequency of anti-skeletal muscle antibodies in non-thymoma patients. Their occurrence was related to the age at onset in patients with an onset of MG beyond 40 years and with duration of disease in patients with an earlier onset of MG. This stresses the heterogeneity of late onset and early onset MG. Serial measurements in individual patients revealed no evidence for a direct thymic role in the generation of AMA. There was no difference in specificity of AMA in early and late onset patients. The presence of AMA in CT-negative patients is not necessarily suggestive for a radiological shortcoming in the detection of a thymoma as in 16 AMA positive patients from our series no thymoma was found at operation or autopsy.

Fluctuations of AMA were studied in relation to clinical changes and fluctuations in a-AChR in **chapter IVc**. Forty-two patients with generalized myasthenia gravis were studied in clinical and serological follow-ups over several years under various conditions. Results from this study demonstrate that AMA fluctuate in strong relation to a-AChR, clinical course and immunosuppressive therapy. Thymomectomy resulted in an increase or first appearance of AMA in ten of the twelve patients without immunosuppressive medication. a-CAE decreased faster than a-AChR after institution of immunosuppressive agents resulting in clinical improvement. Further discordances between a-CAE and a-AChR were rarely found and they did not yield any further information about subsequent clinical course.

A short synopsis of thymic physiology and pathophysiology in MG is given in **chapter Va**. Much evidence for a role of the thymus in the development of self-tolerance has been found in recent studies. Self-antigens present in the thymus may play a role in this process. Notwithstanding the presence of myoid cells with acetylcholine-receptors (AChR) in the normal and also in the MG-thymus, an autoimmune reaction against the peripheral AChR occurs in MG. Much 'peripheral' lymphoid tissue - responding to self-antigen in the thymic medulla ? - is found in the perivascular spaces of the MG-thymus. The basement membrane separating the thymic parenchyma from the perivascular space is found disrupted in MG and an abnormal high proportion of B-lymphocytes is found the medulla. In vitro studies showed that the thymus provides a favourable milieu for differentiation of B lymphocytes and that a-AChR can be produced in a high rate by thymic B-lymphocytes. Proteins with AChR-like features are present in thymic tumors and may induce a cross-reaction against peripheral AChR.

In **Chapter Vb**, histological findings in our own group of 53 non-thymoma patients are described. The resemblance of thymic follicles with peripheral lymph node follicles was confirmed. Diffuse B-cell infiltration (thymitis) in the medulla was found in all thymuses of our series (also in the preparates in which no follicles were found with HE-staining). A high proportion of IgA+ B-lymphocytes and plasma cells was found in MG-thymuses and in addition some MG-thymuses contained large numbers of CD57+ cells. We found no differences in thymic histology between responders and non-responders to TE. No relation between thymic histology and a-AChR antibody levels was found. Like others, we did find a tendency that severe hyperplasia was related to a greater delay of improvement.

The main conclusions of this study are summarized in **chapter VI**. Clinical implications are discussed. Some hypotheses about the role of the thymus in MG and possible mechanisms of TE as therapy for MG are reviewed.